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Original Article

VALIDITY OF ANTI-CCP ANTIBODIES AS MAJOR IMMUNOLOGICAL MARKER OF RHEUMATOID ARTHRITIS PATIENTS IN THI-QARPROVINCE IRAQ

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ABSTRACT

The aim of the study to determine the level of anti- cyclic citrullinated protein antibodies (ACCPA) of rheumatoid arthritis (RA). Establishing profile and to ensure validity of (ACCPA) in diagnosis rheumatoid arthritis. This study including 88 cases, patients as 48 (female 17 and 33 male)with rheumatoid arthritis whose follow- up was carried out at the outpatient clinic of the rheumatology unit Al-Hussein General Teaching Hospital, Thi-Qar province, Iraq. Patients and control were divided into four subgroups based on the characteristic of smoking. The control group include 40 at the same hospital for no inflammatory diseases. We determined the plasmatic level of anti-cyclic citrullinated protein antibodies (ACCPA). In comparison to controls, patients with rheumatoid arthritis presented high concentrations of anti-cyclic citrullinated protein antibodies (ACCPA) with high significant difference (P≤0.001). (determine by plasmatic level), the results also demonstrated the higher concentration found to be in (21-40) age group its mean (0.3670±0.229)(P≤0.001) and the lowest concentrations found be in (1-21) years group, its mean (0.111±0.0215). Also not shown significantly difference between male and female groups. Our results indicate the presence relationship between smoking habit and rheumatoid arthritis and the age range 21-40 more susceptible to be effected.

KEYWORDS: Anti-Cyclic Citrullinated Protein Antibodies(ACCPA), Auto Antibodies, Citrullination, Rheumatoid Arthritis, Smoking

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic persistent systemic inflammatory polyarthritis of unknown cause, mainly affected synovial membrane of multiple joints, principally the peripheral joints in a symmetrical fashion such as those of fingers, resulting in cartilage destruction, bone erosion and joints deformities (Lipsky 2005).

A number of autoantibodies have been described in RA, both their clinical associations and as disease biomarkers, of these only two different groups of autoantibodies – rheumatoid factors (RFs) and anti-citrullinated protein antibodies (ACPA), are used in clinical practice due to their high sensitivity and specificity (Mewar and Wilson., 2006).RFs are present in 60% to 80% of RA patients, but can also be detected in up to 5% of healthy individuals and in a substantial proportion of other systemic diseases such as sjogren's syndrome and many systemic infectious diseases (Steiner and Smolen, 1998).

Citrullination is a common post-translational modification (PTMs) of proteins performing the transformation of a positively charged of the amino acid arginine arginine residue into the neutral the amino acid citrulline (figureure 1). This transfigureuration are a hallmark modify the three-dimensional structure of histone proteins and also generate neo-epitopes on peptides existing by the human leukocyte antigen (HLA) system. Citrullination and methylation impacts numerous cellular pathways, in particular observed by protein arginine methyltransferases (PRMTs).

Aim of the Study

Establishing profile and to ensure validity of (ACCPA) as major immunological marker in diagnosis rheumatoid arthritis

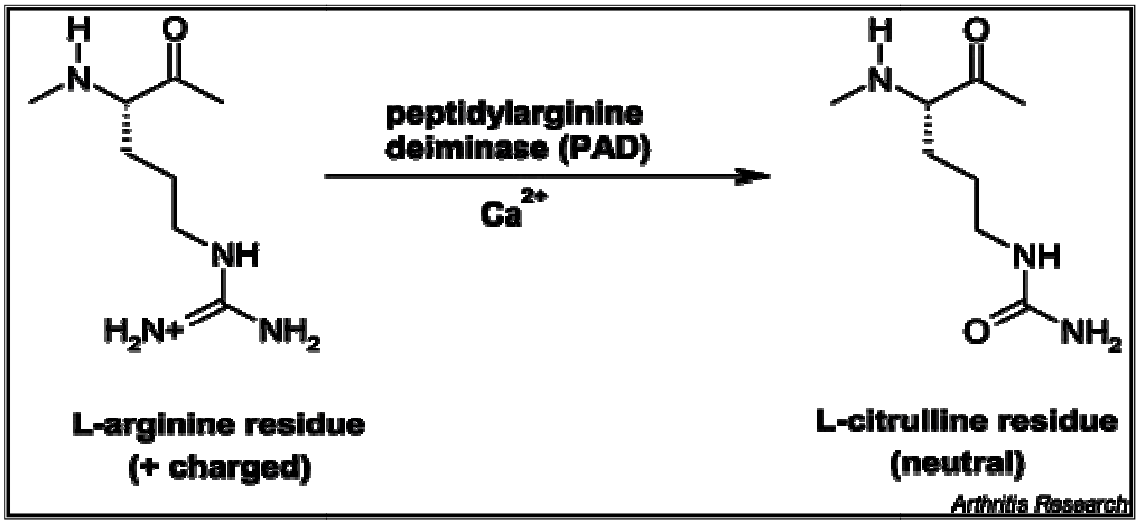

Figure 1: The Enzymatic Conversion of Peptidyl-Arginine to Peptidyl-Citrulline (Van Boekel *Et Al.*, 2002)

It is affected through the enzyme peptidyl-arginine deiminase (PAD) that catalyze cirtillation process by five different patterns because this enzyme contains five isoforms which explicated in a different way in numerous tissues (Vossenaar *et al.*, 2003). Citrullination may also be It urged continuously in the synovium joint membrane of patients with RA under inflammatory disorders especially in smokers categories (Makrygiannakis *et al*., 2008). In figure. 2 which it is shown numerous proteins in the synovium of joint that undertake citrullination such as fibrin, vimentin, fibronectin and alpha-enolase (Van B. *et al*., 2012; Jurgen *et al*., 2015). Molecular modeling data indicate that peptides containing citrulline, but not arginine, can be bound by *0401 MHC molecules. This citrulline-specific interaction might be the basis of a citrulline specific immune response (Hill *et al.*, 2003). These data suggest that the specific structure of HLA-DR4 molecules plays an important role in the induction of autoimmunity to citrullinated proteins. Although there is not an absolute requirement for HLA-DR4 in order to develop anti-CCP antibodies. In predisposed characters a hurt of immune tolerance to citrullinated proteins and peptides possibly will progress because T cells in the periphery are threatened by peptide-HLA complexes against which they have not been polarized in the thymus gland. T cells from ACPA+RA patients diagnose citrullinated vimentin peptides *in vitro* controlled by HLA-DR4 and comparable outcomes have been prepared in HLA-DR4 transgenic rats (Feitsma *et al* ., 2010).

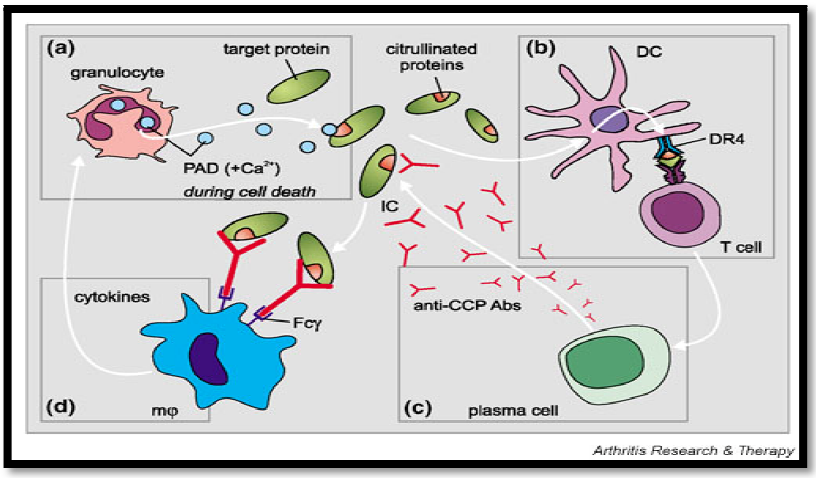

Figure 2: Possible Links between Rheumatoid Arthritis (RA) Specific Anti-Cyclic Citrullinated Peptide (Anti-CCP) Antibodies and RA-Associated Genetic Factors (A)PADI4 Single Nucleotide Polymorphisms (Snps) May Lead to Elevated PAD4 Expression and to Increased Citrullination of Proteins (B) RA-Associated HLA-DR4 Molecules (DR4) Can Bind and Present Citrullinated Peptides Much More Efficiently than Non Citrullinated Peptides (C) IL-6 Promoter Snps are Associated with Increased Anti-CCP Antibody Production and Severity of the Disease (D) Various Cytokine Polymorphisms are Associated with RA and May Lead to Stronger Effects of Immune Complex Activated Cells. ABS, Antibodies; DC, Dendritic Cell; Fcγ, Fcγ Receptor; IC, Immune Complex; M Ψ, Macrophage; PAD, Peptidylargininedeiminase. (Feitsma*et Al* ., 2010)

Evidence shows that auto reactive B-cells can be driven by the T-cells to produce immunoglobulin G (IgG) such as auto antibodies ACPA after non-specific stimulation *in vitro* that may be directly involved in joint damage and B-cells are known to be critical in activating CD4 cells (Kotzin, 2005; Bellatin *et al*., 2012) (figure 2). B cells beginning around one third of normal fragments carrying these HLA-DR alleles, but without family history of RA, besides created ACPA antibodies *in vitro*. (Feitsma *et al*., 2010).

MATERIALS AND METHODS

The samples of the study includes88 cases, 48 patients with RA. They were achieved four or more of the criteria of the 2010 American College of Rheumatology. These patients entered the Al Husain Teaching Hospital at Al-Nasiriyah city, Iraq. During the period between October 2015 and February 2016. The diagnosis of these patients were performed under supervision of group of rheumatologists. The sample of the study include 40 person apparently healthy volunteers were included in this study. Take 2 ml of serum from each case and use ELASA– kit supply from Human company – Germany to measure the concentrations levels of anti-cyclic citrullinated protein antibodies (ACCPA).

RESULTS

Through reliance on the results found in the table blew, it is found that there is a high concentration in the patients group of smokers, its mean (0.6893±0.623) compared with other groups (0.1968±0.1488), (0.0967 ± 0.0145) and (0.0968±0.0252) respectively.

Also note there is a significant difference between in the group (patients smokers P≤ 0.00) compaerd with other all studied groups at signficant level (0.05)

Table 1: Concentration of ACCP (RU/ ml) between the Studied Groups Depending on Smoking Habit

Group	No.	% of No	Mean	P Value
Patients Smokers	**26**	**29.5%**	**.6893±.623**	**Hs**
patients non-smokers	**22**	**25.0%**	**.1968±.1488**	
Healthy smokers	**20**	**22.7%**	**.0967±.0145**	
Healthy non-smokers	**20**	**22.7%**	**.0968±.0252**	
Total	**88**	**100.0%**	**.2936±.2417**	

HS= High Significant difference (P≤0.001).NS= Non Significant difference(P>0.05)

Table 2: Concentrations of Anti -CCP (Ru / Ml) Between The Studying Groups Depending on Age Group

Group	No.	Mean±Sd of Accp Level	%of No	P Value
(1-20) age group	**10**	**.1111±.0215**	**20.8%**	
(21-40) age group	**20**	**.3670± .2 29**	**41.6%**	
(41-60) age group	**9**	**.3574±.28106**	**18.7%**	**HS**
(>60) age group	**9**	**.2624±.250**	**18.7%**	
Total	**48**	**.2929± 295**	**100.0%**	

(Sig=0.379). (df= 3) .(F 1.051)

HS= High Significant difference (P<0.001).NS= Non Significant difference(P>0.05).

While not record any other high significant differences between the groups but we can show increase in concentration in(patients non-smoking (.1968±.1488))higher than the healthy groups (.0967±.0145),(.0968±.0252).

Table 3: Multiple Analysis Between Studied Groups According the Age Group to Evaluated Concentration of ACCP

(I) Group	(J) Group	No.	Mean Difference (I-J)	Sig.	Df	P Value
patients smokers	patients non-smokers	22	.4924*±.1037	0.00	3	HS
	Healthy smokers	22	.5925*±.1037	0.00	3	HS
	Healthy non-smokers	20	.6068*±.1065	0.00	3	HS
patients non-smokers	patients smokers	26	-.4924*±.1037	0.00	3	HS
	Healthy smokers	22	.1001±.1079	.356	3	NS
	Healthy non-smokers	20	.1143±.1106	.304	3	NS
Healthy smokers	patients smokers	26	-.5925*±.1037	0.00	3	HS
	patients non-smokers	22	-.1001±.1079	.356	3	NS
	Healthy non-smokers	20	.0142±.1106	.898	3	NS
Healthy non-smokers	patients smokers	26	-.6068*±.1065	0.00	3	HS
	patients non-smokers	22	-.1143±.1106	.304	3	NS
	Healthy smokers	22	-.01422±.1106	.898	3	NS

The mean difference is significant at the .05 level.

HS or **= High Significant difference (P<0.001) S or *= Significant difference (P≤0.05). NS= Non Significant difference (P>0.05).

The results also demonstrated in the table 2, we can show according age groups, the higher concentration found to be in (21-40) age group (.3670± .229) and the lowest concentration in (1-20) age group.1111±.0215), comparison between all age group, we were not found significant difference negotiable calibration. In table4, the result also showed that in

female patients, (0.297±0.2020) anti-CCP concentration found significantly higher in the male group (0.290±0.2980), but not show significantly difference between them.

Table 4: Concentration of Anti -CCP (RU/ML) Between the Studied Groups Depending on the Gender

Groups	No.	%of No.	Mean ± Sd	P Value
Male	31	64.5%	.2904±.29	NS
Female	17	35.4%	.2978±.20	
Total	48	100.0	.292±.29	

(Sig =0 .951). (**df= 46**).(**t = -0 .061**)

HS= High Significant difference (P<0.001).

NS= Non Significant difference (P>0.05).

DISCUSSIONS

This study demonstrated that there is clear significant difference between the patients group and control group as clarified in the tables 1 and 2, which in it we subdivided group into smokers and no smokers, this results goes in correspondence with (Abdullah 2010;Ruyssen-Witrand *et al*.,2012), and this happens due to the following reason, it was noticed that have there no significant difference between male and female groups but there is noticeable increase and as clarified in following table 4.

It was noticed by dividing groups of patients according to age classes that there is no significant difference between age classes but we can show the higher concentration found in (21-40) age group) and the lowest concentration in (1-20) age group). By used Statistical analysis test ANOVA to comparison between all age group as in table3.Recently a highly specific autoantibody system has been described for RA, in which patients develop antibodies to citrullinated, and this has resulted inthe development of the anti-cyclic citrullinated peptide (anti-CCP)antibody test (Quinn *et al*., 2006).

Anti-CCP antibody which has been detected in the sera of the RA patients. Anti-CCP Antibodies showed high significant in RA in comparison with control groups (P≤0.001), as summarized in table 2.

Anti-CCP was a good serological marker for RA should be highly specific for the disease and be able to distinguish RA from other arthritis that mimic RA. The frequency of anti-CCP positivity among the RA patients sera (69%), this percentage is higher than that of (Khosla *et al*., 2004) and (Nell *et al*. ,2003) who proposed 50-60 % detection of anti-CCP in RA cases, while, the present study comparable to Iraqi study is (68.09%) (Tofiq, 2007). Additionally, there is a highly significance in RA and control groups (P<0.001). This result agrees with these studies, which had positivity reached to (68%) (Quinn *et al*., 2006) and indicated the anti-CCP which was a good marker for RA disease. In many early cases of RA, clinical symptoms are mild and nonspecific, and patients will not fulfill ACR classification criteria for RA. Therefore, the detection of a disease-specific autoantibody like anti-CCP could be of great diagnostic and therapeutic importance . Anti-CCP antibodies may be detected in roughly 50-60% of patients with early RA at baseline (e.g., at their initial encounter with a specialist, usually after 3-6 months of symptoms) because it can often be detected before complete clinical manifestation of the disease .The specificity of anti-CCP is around 95-98% as it regards undifferentiated forms of arthritis that do not develop into RA. The use of anti-CCP results in the decision whether a patient should be treated aggressively at an early stage or not is an important area for research. In addition, the relationship of levels of anti-CCP antibodies and various therapeutic interventions is under investigation.

CONCLUSIONS

Anti-cyclic citrullinated protein antibodies (ACCPA) represent a superior serological marker for Rheumatoid arthritis, so that (ACCPA)is :

- High frequency of anti-CCP positivity for RA.
- Able to differentiate RA from other kinds of Rheumatic Disease or imitative RA.
- Anti-CCP which was a good marker for RA disease.
- Great diagnostic and therapeutic importance.
- Facilitate in predicting disease progress.

Its prognostic potential may aid the rheumatologist in reaching decision with a reproducible and easily performed ELASA technique.

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